JUN 25 2004  
TECH CENTER 1000DATA SHEETIntroduction

It is well known that solar radiation is the primary cause of premature ageing and skin cancer. The action of UV generates free radicals in the skin, which damage DNA, protein and cell membranes. As a result, discolouration, mottling and precancers can occur.

Skin is exposed externally and internally to oxygen. Chemicals, radiation and drugs may activate the oxygen to yield reactive oxygen species (ROS) or free radicals, which can initiate a chain reaction.

Many different consequences of such a chain reaction all contribute to ageing. Firstly the cross linkage of collagen causes thin inflexible wrinkled skin. Secondly the breakdown of hyaluronic acid diminishes the water holding capacity and finally free radicals attack phospholipids in the cell membrane thereby getting access to DNA and enhancing the risk of skin cancer.

1. In Vitro Measurements of lipid oxidation

Measurements were carried out in accordance with the method described on pages 50-51 of PCT/EP00/08729.

Results

The results are expressed as the % inhibition of free radical mediated lipid peroxidation compared to that achieved using the vehicle alone. Data for individual antioxidants are shown in Table I.

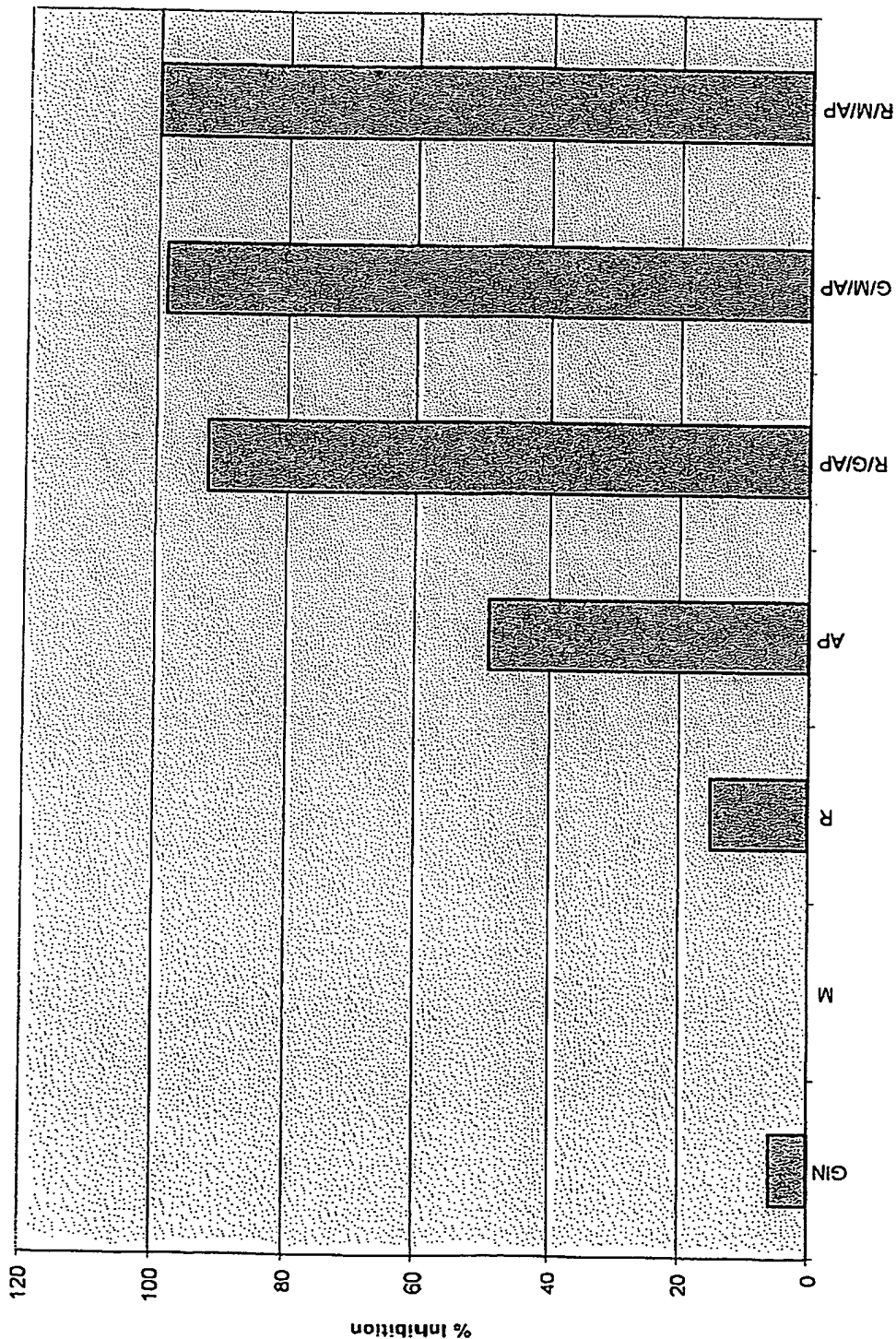
Table I

Antioxidant	% inhibition
Morus alba (m)	0
Origanum vulgare (or)	29
Rosmarinus officinalis (r)	15
Panax ginseng (gin)	5.7
Grape seed (gs)	0
Ascorbyl phosphate (ap)	49
Ascorbic acid ester (aae)	48

The results obtained for combinations of three antioxidants (at the same total antioxidant concentration as for the individual antioxidants) are shown, in comparison with the individual components, in Graphs I, II and III.



SYNERGISTIC COMBINATIONS OF THREE ANTIOXIDANTS GRAPH I



Peroxidation

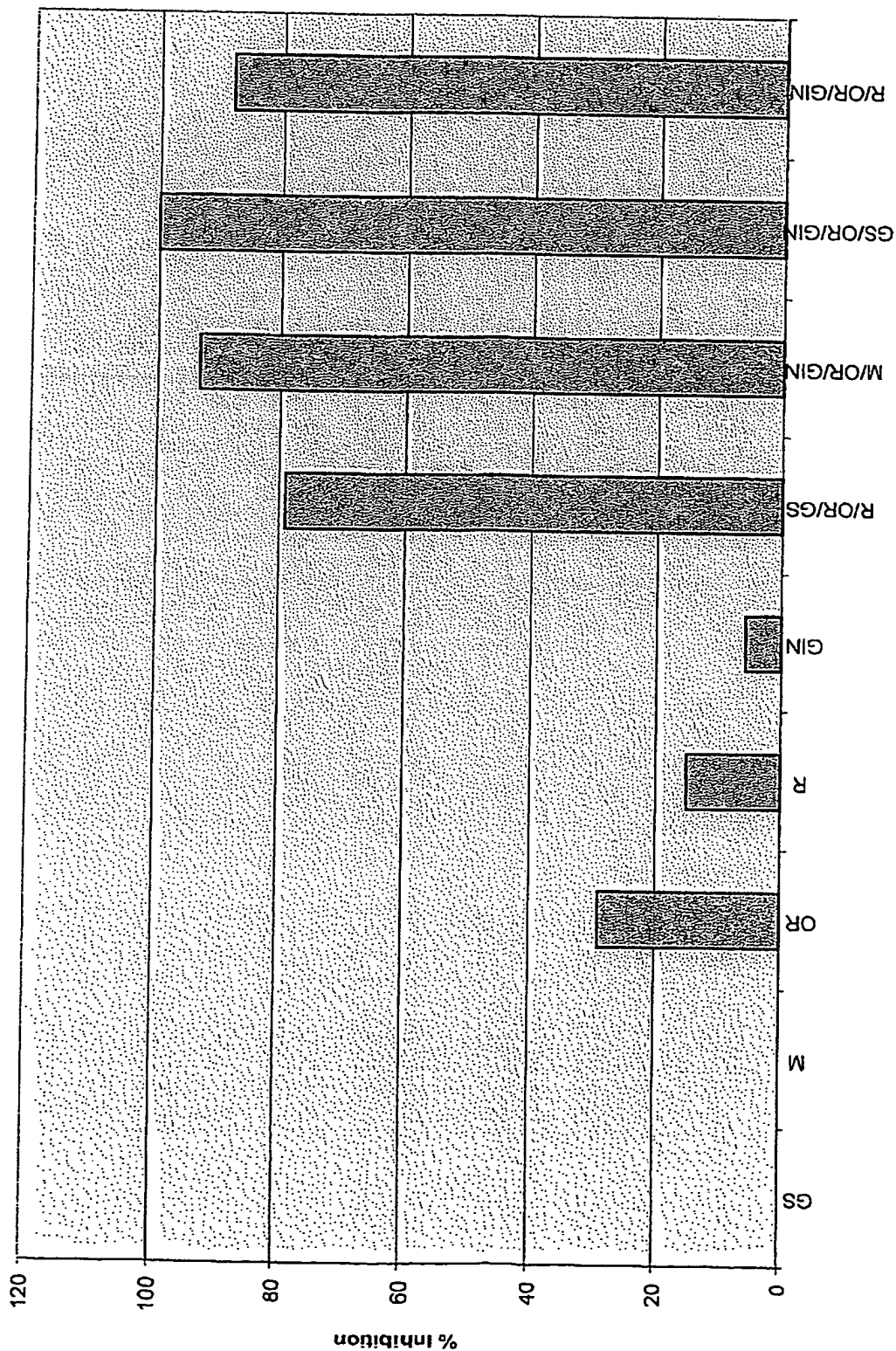
GIN - Ginseng  
M - Mulberry  
R - Rosemary  
AP - Ascorbyl Phosphate

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SYNERGISTIC COMBINATIONS OF THREE ANTIOXIDANTS GRAPH II



Peroxidation

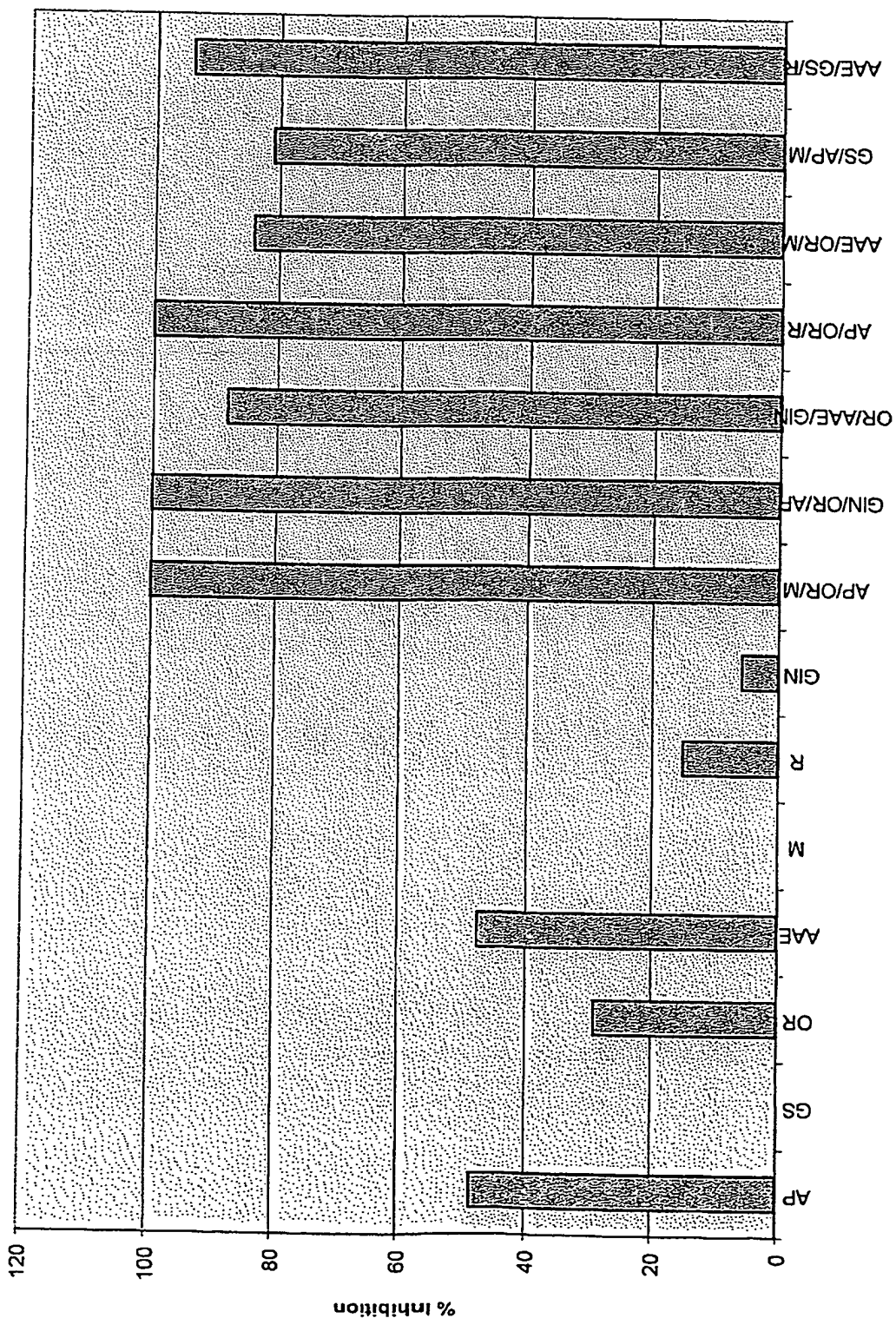
GS - Grapeseed  
M - Mulberry  
OR - Origanum  
R - Rosemary  
GIN - Ginseng

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SYNERGISTIC COMBINATIONS OF THREE ANTIOXIDANTS GRAPH III



Peroxidation

AP - Ascorbyl Phosphate  
 GS - Grape seed  
 OR - Origanum  
 AAE - Ascorbic Acid Ester  
 M - Mulberry  
 R - Rosemary  
 GIN - Ginseng

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Further measurements were made, in a separate series of experiments, and the results shown in Table II were obtained:

Table II

Combination	Predicted % inhibition	Actual % inhibition
ap/m/or	78	100
ap/m/gin	54.7	98.6
ap/m/gs	49	81
ap/gin/r	69.7	92
ap/m/r	64	99.9
or/m/gin	34.7	93
or/gin/gs	34.7	100
r/or/gin	49.7	88
ap/or/gin	69.7	100
or/aae/gin	48	88
aae/gs/r	63	94

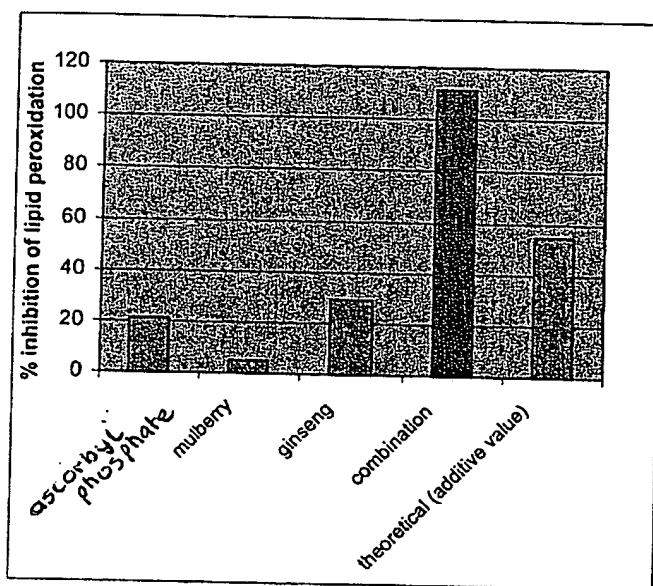
### Summary

All of the following combinations of three antioxidants have been shown to be synergistic, ie the % inhibition achieved with these combinations is considerably in excess of that predicted on the basis of addition of the results for the individual components:

Rosmarinus officinalis / Panax ginseng / Ascorbyl Phosphate  
 Panax ginseng / Morus alba / Ascorbyl phosphate  
 Rosmarinus officinalis / Morus alba / Ascorbyl phosphate  
 Morus alba / Origanum vulgare / Panax ginseng  
 Grapeseed / Origanum vulgare / Panax ginseng  
 Rosmarinus officinalis / Origanum vulgare / Panax ginseng  
 Ascorbyl Phosphate / Origanum vulgare / Morus alba  
 Panax ginseng / Origanum vulgare / Ascorbyl Phosphate  
 Origanum vulgare / Ascorbic acid ester / Panax ginseng  
 Ascorbyl phosphate / Morus alba / Grape seed  
 Ascorbic acid ester / Grape seed / Rosmarinus officinalis  
 Rosmarinus officinalis / Origanum vulgare / Grape seed  
 Ascorbyl phosphate / Origanum vulgare / Rosmarinus officinalis  
 Ascorbic acid ester / Origanum vulgare / Morus alba

## 2. In vivo benefits of combination of antioxidants

The degree of protection afforded against lipid peroxidation (free radical damage) was measured by the method outlined at page 52, lines 10-17 of US SN 10/069,975. A comparison was made between the effects of Magnesium ascorbyl phosphate, mulberry (morus alba) and panax ginseng alone, and the combination of all three antioxidants. The results are shown in the graph below:



The graph shows that when used singly in the formulation, the antioxidants provide limited protection against lipid peroxidation. When combined, there is a synergistic, protective effect. The theoretical additive effect is also shown for comparison. This is the predicted protection that should be expected by combining the single antioxidants. It can be seen that it is considerably less than the experimental result, confirming the synergistic effect.